Metabolic dynamics in the unicellular diazotrophic cyanobacterium *Cyanothece* sp. PCC 7822 across a 12 hour light-12 hour dark cycle under nitrogen fixing conditions

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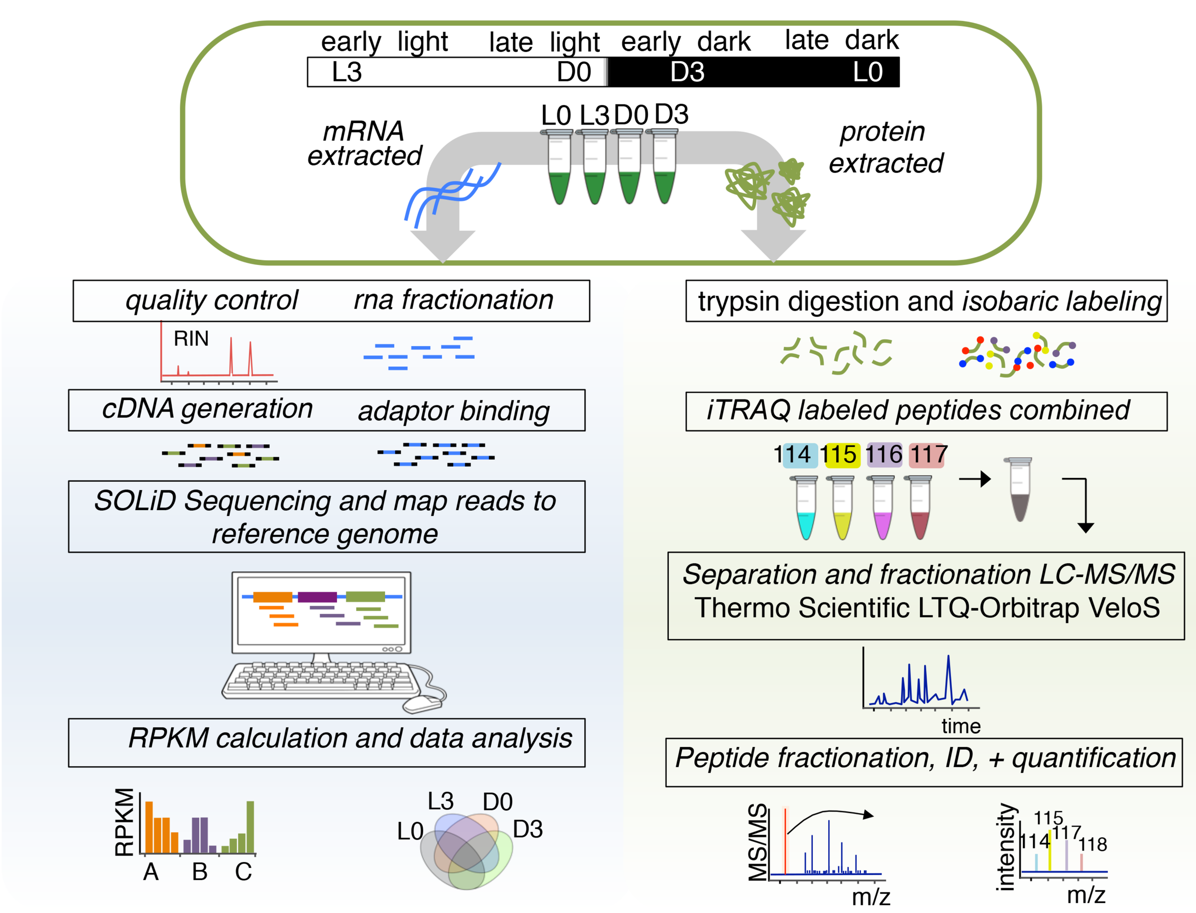
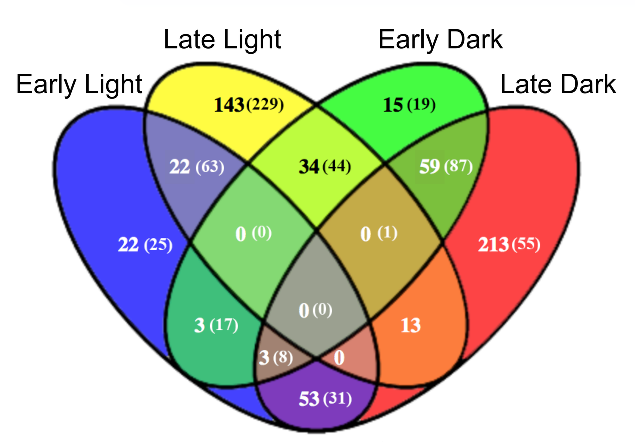
Background

Cyanothece sp. PCC 7822 is an excellent cyanobacterial model organism with great potential for biotechnology applications. Utilizing transcriptomic and proteomic methods, we quantified the relationships between transcription and translation underlying central and secondary metabolism in response to nitrogen free conditions across a 24 hour period consisting of 12 hour light and 12 hour dark.





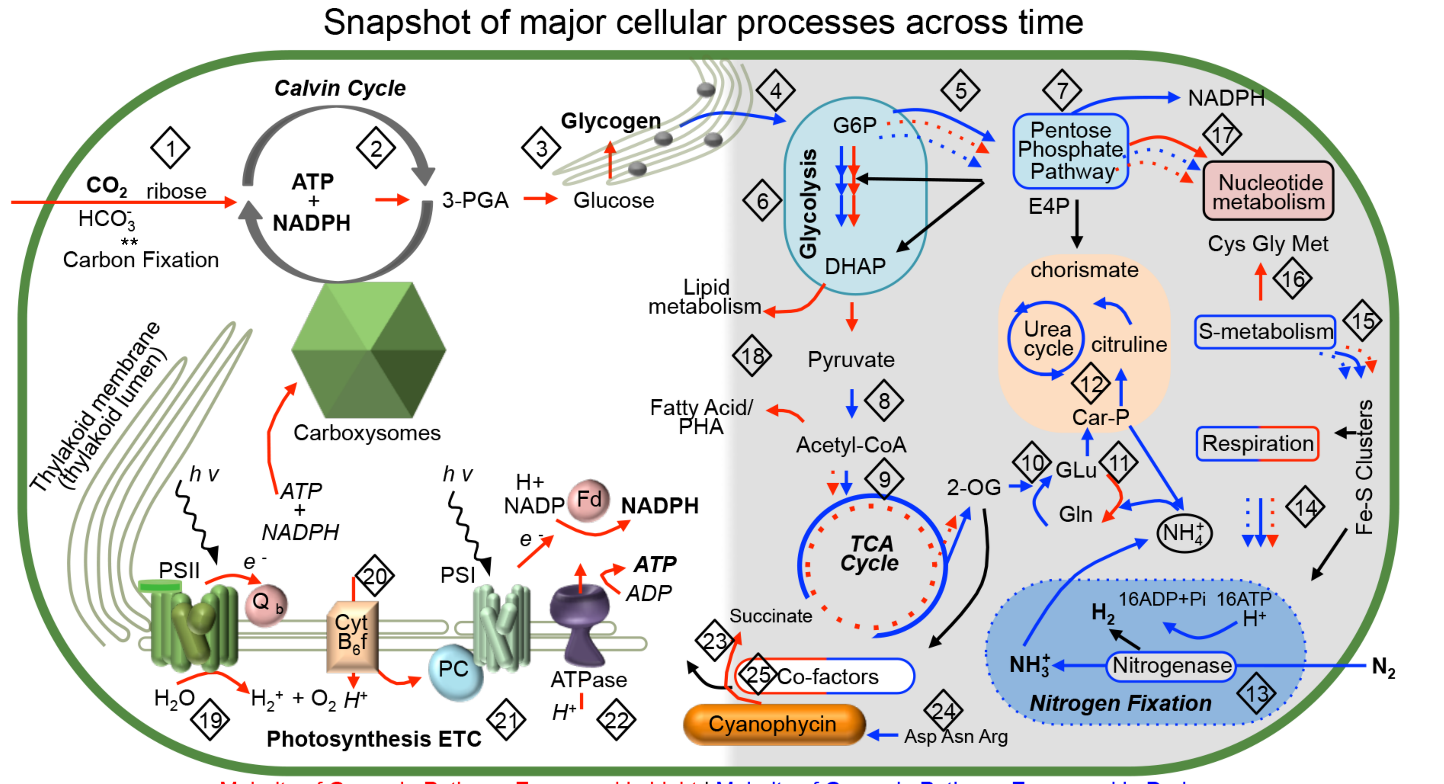
# of genes significantly up (>2 FC) (bold) and down (parentheses) regulated (<0.5 FC) (p-vlaue >0.05)

We quantitatively measured a total of 6766 mRNAs and 1322 proteins at four time points across a 24 hour light-dark cycle.

Prior to the transition into the light and dark periods, we see the highest number of differentially expressed genes consistent with the understanding that the cell needs to substantially adjust its enzymatic repertoire to meet the different needs during the light and dark periods.

Transcription of important metabolic functions typically began in a light period prior to their maximum expression. Interestingly, initiation of transcription of genes encoding some key metabolic functions is not restricted to the light or to the dark exclusively.

Snapshot of major cellular processes across time

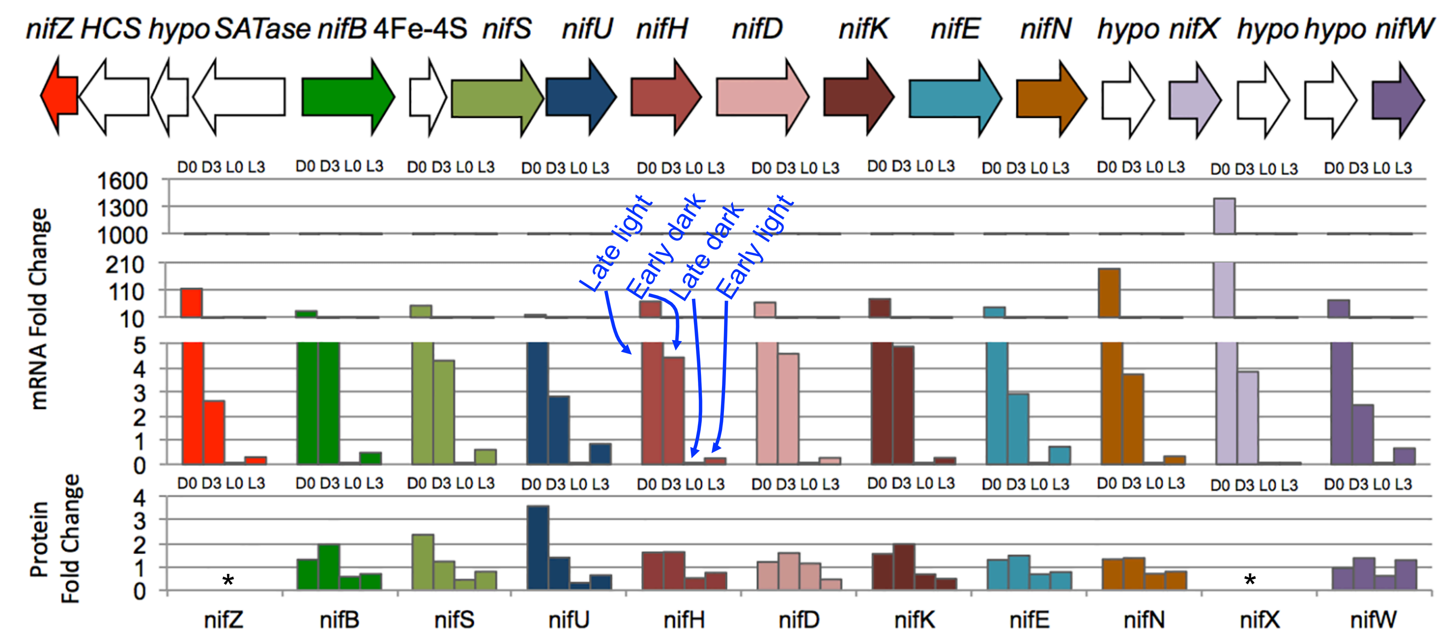


Majority of Genes in Pathway Expressed in Light | Majority of Genes in Pathway Expressed in Dark

mRNA protein mRNA protein

**No dotted arrow indicates that protein and mRNA expression dynamics similar

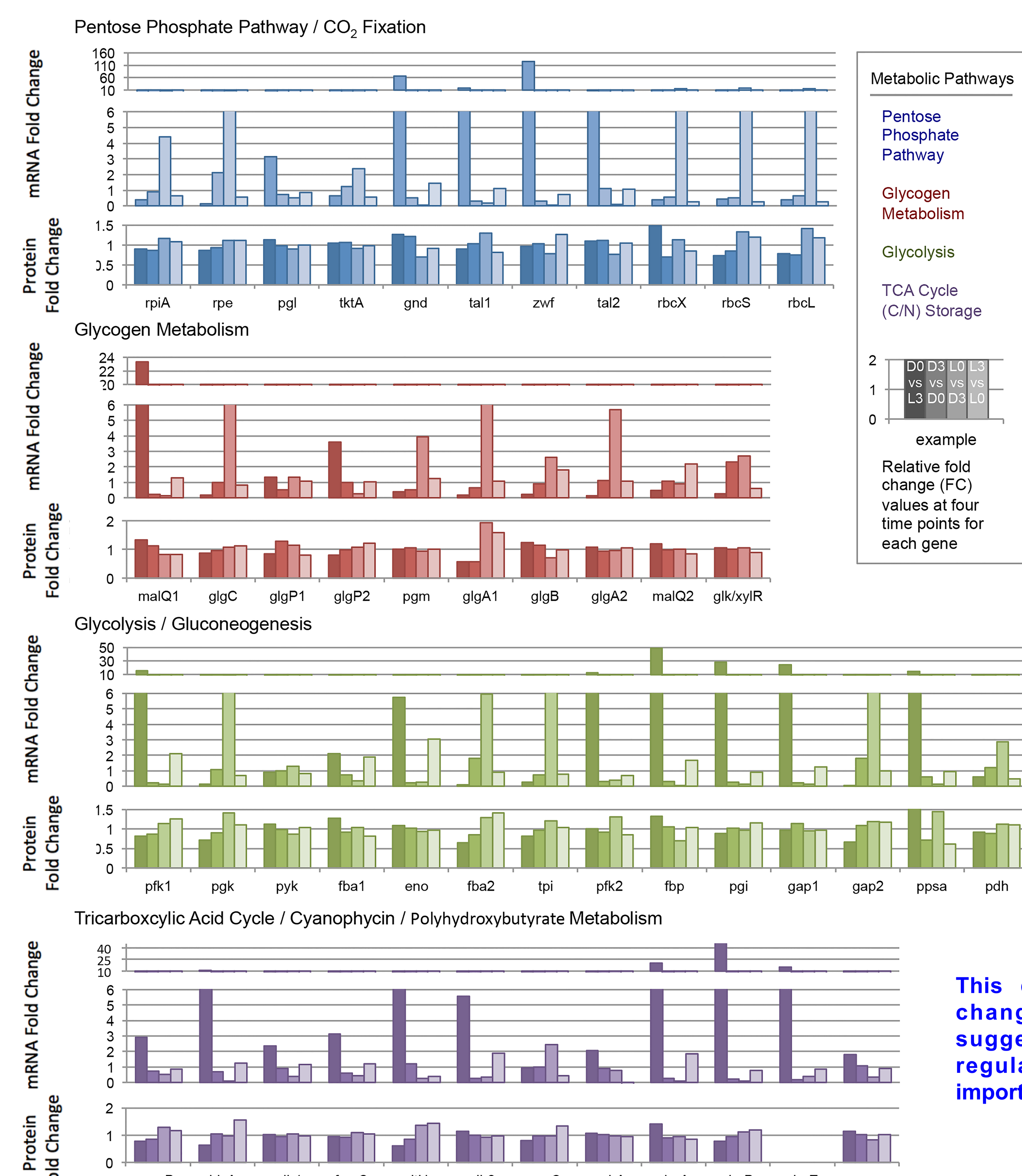
Central Metabolism Protein and mRNA Dynamics



Nitrogenase expression occurs strictly during late light/early dark

All genes in the *nif* cluster show in-phase mRNA and protein expression dynamics

* No protein detected in analysis



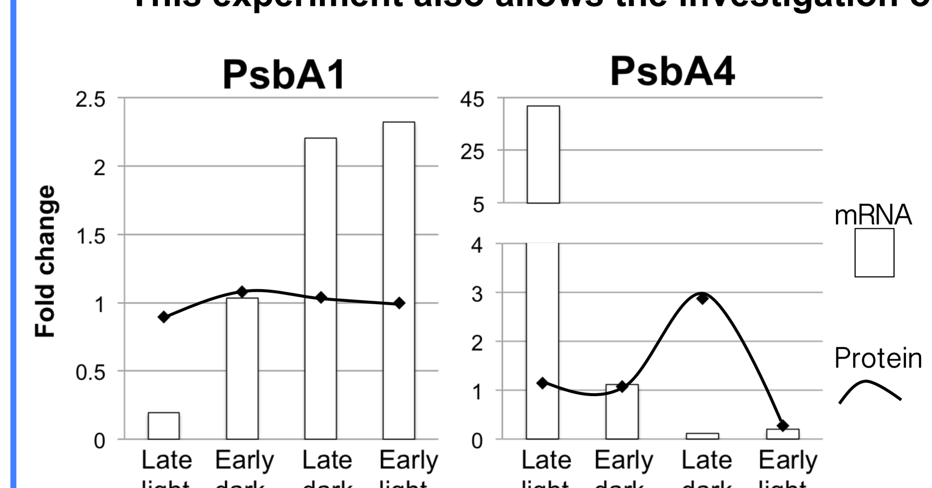
Differential transcription across time points varied, ranging from modest to extreme changes between time points

This differed from changes in protein abundances, which changed very little in most cases.

In some instances, changes at the transcriptional level had little to do with the abundance of the proteins

This difference in the abundance changes of mRNA and protein suggests that post-translational regulatory mechanisms play an important role in *Cyanothece* 7822

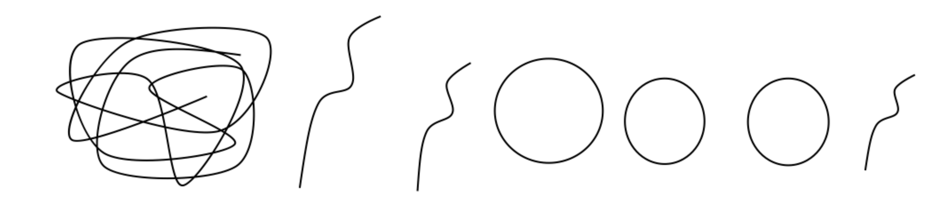
This experiment also allows the investigation of differential expression of multi-copy gene families



- PsbA isoforms were differentially expressed across the light and the dark.
- PsbA1, was upregulated in the late dark/early light with mRNA levels ~5-6X higher than all others at all time points.
- **PsbA4 was upregulated in the late light period with peak protein abundance during the dark period, suggesting that this gene encodes the sentinel D1 protein.**
- Other multi-copy genes were similarly expressed during different time points.

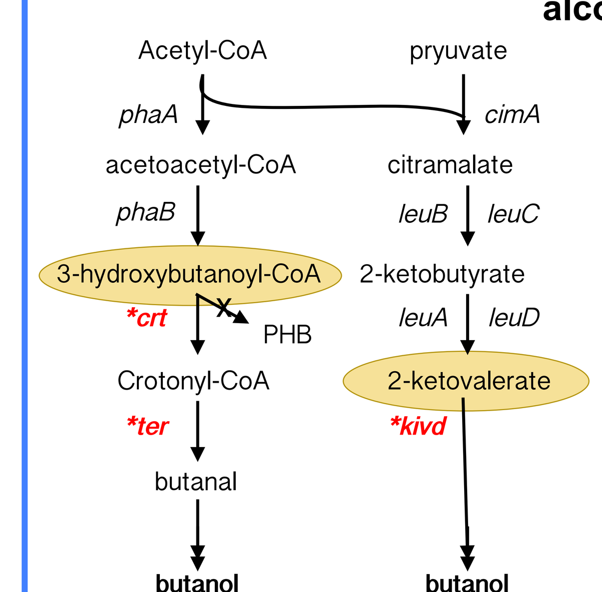
Expression of genes on extrachromosomal plasmids and pathways relevant to for generating high-value products

Cyanothece sp. PCC 7822 has the largest genome (shown below) of the *Cyanothece* strains and has 6 extrachromosomal plasmids, three linear and three circular. All of these strains were sequenced by JGI and the results are summarized in Bandyopadhyay A, *et al.* (2011). *Mbio* 2(5).



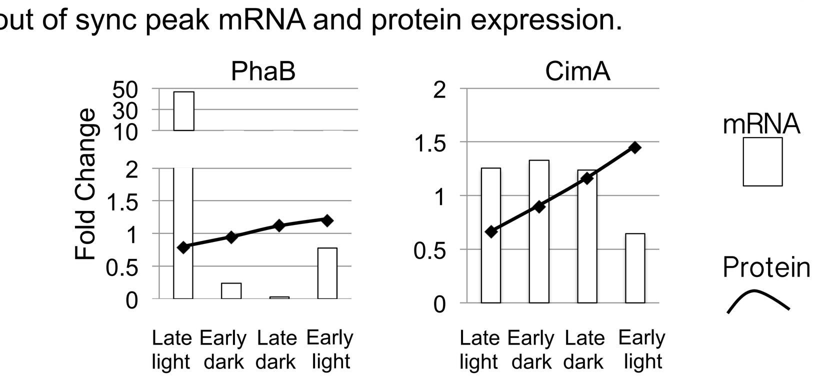
Genomic Element	6.1 Mb	879 kb	473 kb	291 kb	47 kb	43 kb	13 kb
Proteins Expressed	1036	53	7	1	3	1	1
Total Genes on Element	5663	595	422	280	32	36	13
% Genes Expressed	18	9	1.5	0.5	9	3	7.5

Cyanothece 7822 has the potential to produce various biofuels like terpinoids, alcohols, and fatty acids



Crt = 3-hydroxybutyryl dehydrogenase
Ter = trans-2-enoyl-CoA reductase

Cyanothece 7822 has two potential pathways for producing butanol. Peak protein expression for both branches occurs predominantly during the light phase. PhaA and CimA display out of sync peak mRNA and protein expression.



This is a good example of how important dual transcriptomics and proteomic investigations are to fully understanding a given cyanobacterial strains metabolism.

Conclusions

- The expression of genes related to photosynthesis, nitrogen fixation, and carbon storage, were typically up-regulated late in the preceding light or dark period, correlating with the fact that nitrogenase was active in the late light period.
- In glycolysis, transcriptional expression levels had little to do with the abundance of the proteins at various times throughout the diurnal cycle. In the pentose phosphate pathway, the mRNA changes are far more striking than those of the proteins. This pathway is used in both the light and the dark and, although the transcripts of many proteins were most abundant in the dark, the protein levels remained high throughout much of the 24h period.
- Interestingly, transcription of proteins also used for CO_2 fixation was highest in the late dark, thus preparing the cells for the onset of light-driven photosynthesis. The TCA cycle followed a similar pattern, although the proteins seemed to be at slightly higher abundance in the light.
- Glycogen/starch metabolism is also represented by a number of different relationships where the proteins remained more stable than the mRNA levels would have indicated.

This investigation of concurrent transcriptional and translational activity within *Cyanothece* sp. PCC 7822 provides quantitative information of metabolic pathways relevant to engineering efforts. The identification of expression patterns for both mRNA and protein provides a basis for improving biofuel production in this strain generating a improved metabolic model. Expression analysis of the genes encoded on the 6 plasmids provided insight into the possible acquisition and maintenance of some of these extra-chromosomal elements.

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